Changes in Microbial Community Structure During Biostimulation for Uranium Reduction at Different Levels of Resolution

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a National Science Foundation Engineering Research Center in the MSU College of Engineering

ABSTRACT

Former radionuclide waste ponds at the ERSP-Field Research Center in Oak Ridge, TN pose several challenges for uranium bioremediation. The site is marked by acidic conditions, high concentrations of nitrate, chlorinated solvents, and heavy metals. Above-ground treatment of groundwater, including nitrate removal via a denitrifying fluidized bed reactor (FBR) pre-conditions the groundwater for subsurface uranium immobilization. A series of re-circulating wells serve to create a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (i.e., ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 and FW-102 are the inner zones of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively, which are the outer protective zones. Bacterial community composition and structure of groundwater from the wells were analyzed via clonal libraries of partial SSU rRNA gene. Both qualitative and quantitative methods were used to analyze the changes in bacterial diversity and distribution. LIBSHUFF analysis was used for the comparison of bacterial community population between the different clonal libraries. Bacterial community from the denitrifying FBR was different from the groundwater bacterial community, which indicated that different bacterial communities were stimulated in the two separate systems. The clonal libraries of the re-circulating wells showed that over each phase of manipulation for uranium immobilzation, the bacterial communities of the inner zones of biostimulation were more similar to each other than those of the outer protective zones. The outer protective zones were more similar to the injection well. Clonal libraries from FW-104 (injection), FW-101 and FW-102 showed that bacterial communities of the three wells were initially similar but developed changes through time. FW-101 and FW-102 bacterial communities developed changes in parallel, while those of FW-104 showed gradual change. These results were further compared to data generated from Unifrac analysis. Preliminary results with Unifrac analyses showed that the bacterial community in each of the wells developed changes during the bioremediation process, and the changes could be attributed to the variations along temporal, spatial, and geochemical scales. Diversity indices showed that bacterial diversity tended to increase during the initial phase of uranium bioreduction and decreased toward the end of uranium bioreduction (i.e., low U(VI) levels). As uranium levels declined, increasing *Desulfovibrio* and *Geobacter*-like sequences were detected from the clonal libraries, and the *Desulfovibrio*-like sequences predominated over time. The results were further confirmed via qPCR and the results correlated with OTU distributions for *Desulfovibrio*. The results indicated that the bacterial community composition and structure changed upon stimulating for uranium bioreduction conditions, and that sequences representative of sulfate-reducers and metal-reducers were detected in wells that displayed a decline in U(VI). Further analysis is underway to determine the relationships between different functional groups and site geochemistry.

INTRODUCTION

Uranium is a major groundwater contaminant at the U.S. Department of Energy (DOE) NABIR Field Research Center (FRC) on the DOE Oak Ridge Reservation in eastern Tennessee. The sites are also characterized by acidic conditions (pH 3.5), high concentrations of nitrate (up to 160-200 mM), various heavy metals, and other contaminants. A two-phased approach is currently being used at the FRC to deal with these conditions. The first phase includes neutralization of the groundwater pH and aboveground removal of nitrate, chlorinated solvents, Ca and Al. The second phase involves recirculation of groundwater supplemented with electron donor to stimulate microbial growth, including denitrification of residual nitrate and uranium reduction *in situ.* A series of re-circulating wells establish a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 is the center of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively. The purpose of this study is to evaluate the changes in microbial community composition as conditions are stimulated for uranium bio-reduction in the subsurface.

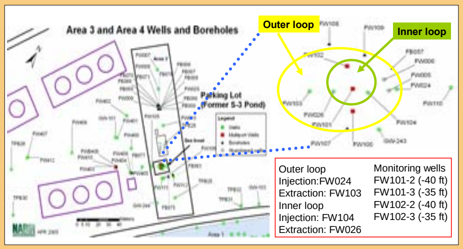

Figure 1. A series of re-circulating wells establish a subsurface bioreactor to stimulate microbial growth for *in situ* U(VI) immobilization. Well FW-104 is the injection well for the electron donor (ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 is the center of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively.

RESTULTS

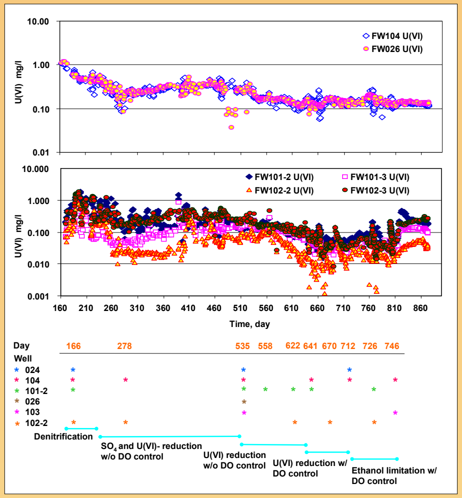

Figure 2. U(VI) concentrations in injection and extraction wells. Samples were analyzed according to uranium levels in the subsurface at different days. *Day 9-136 Clean Water Flush:* Nitrate, aluminum, and calcium were removed to favorable levels for bioremediation. *Day 137-184 In situ Denitrification:* Nitrate was further reduced by stimulation of denitrification by adding ethanol. *Day 185-712 In situ U(VI) Reduction:* Uranium concentrations in monitoring wells were reduced to below 30 μg/L. *Day 712-756 Testing the Stability of Uranium Immobilization:* The test was performed by maintaining recirculation between wells without addition of ethanol for 41 days.

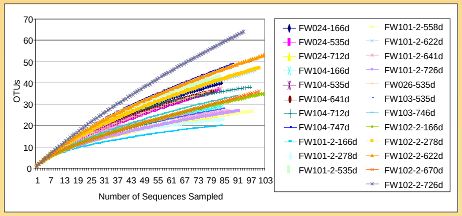

Figure 3. Rarefaction curves constructed with 3% OTU definition. Bacterial communities were analyzed via clonal libraries of the partial SSU rRNA gene. OTUs were calculated by generating a distance matrix in MEGA version 3.1 and importing into DOTUR.

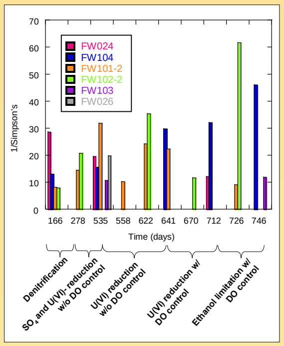

Figure 4. Diversity of each well. The diversity index, 1/Simpson's was calculated by generating a distance matrix in MEGA version 3.1 and importing into DOTUR. The diversity in the injection well, FW104, continued to increase, while fluctuations in diversity occurred in the two bioreduction wells, FW101-2 and FW-102-2, during the bioremediation process. The two outer wells, FW103 and FW026, which were not stimulated for bacterial growth did not experience much change in diversity. A decline in bacterial diversity was observed in the control well, FW024.

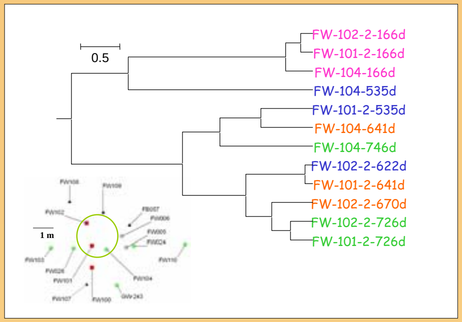

Figure 4. Spatial and temporal variation in the inner loop during biostimulation. Library shuffling (LIBSHUFF) analysis computes the homologous and heterologous coverage within and between clone libraries (http://www.arches.uga.edu/~whitman/libshuff.html). The bacterial community of the two treatment wells and the injection well were compared with LIBSHUFF and the values were used to construct a UPGMA tree. The treatment wells and the injection well were closely related early in the experiment, but diverged post-treatment. The treatment wells were more closely related as treatment time increased.

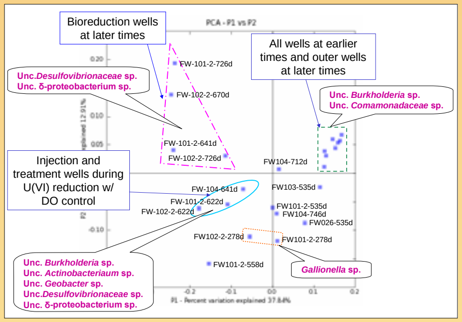

Figure 5. Principal coordinate analysis of the samples performed by UniFrac. A phylogentic tree based upon clonal sequences was exported and each sequence was labeled with sample designations. UPGMA clustering and principal coordinate analysis were performed using UniFrac (http://bmf.colorado.edu/unifrac/index.psp). Samples are represented by squares. The percentages in the axis labels represent the percentage of variation explained by the principal coordinates. The results are in accord with LIBSHUFF analysis (Fig. 4). Shifts in bacterial populations for each sample were determined by lineage-specific analysis in UniFrac.

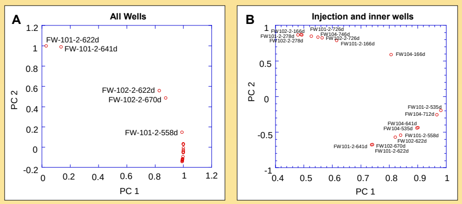

Figure 6. Factor analysis of patterns in environmental physicochemical variables during the bioremediation period. (A) Trends in all of the wells. (B) Trends in the injection and inner wells. The analysis showed that all of the wells started with similar physicochemical variables and changes were observed during the bioremediation period. In particular, the two bioreduction wells developed similar trends in change. Eventually, however, despite the divergence that had occurred during bioremediation, the physicochemical variables in all of the wells shifted again towards the same direction when addition of electron donor was stopped.

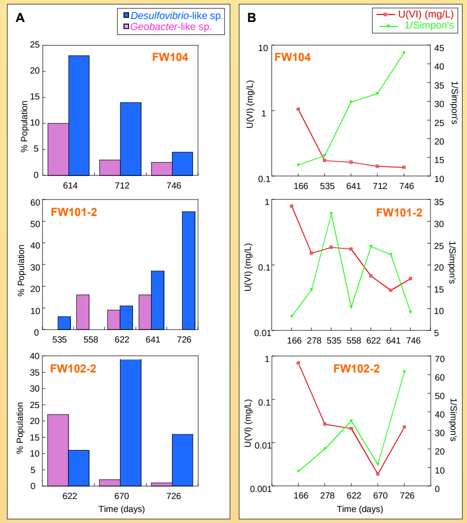

Figure 7. Population and diversity changes in relation to uranium levels in the injection well and bioreduction wells. (A) Percent population of *Desulfovibrio* and *Geobacter* spp. (B) Changes in uranium levels and bacterial diversity. The uranium (VI) levels in the injection well declined at a slower rate compared to the bioreduction wells. *Desulfovibrio-like* and *Geobacter*-like sequences were observed in the injection well, but at later times compared to the bioreduction wells. Declining uranium (VI) levels corresponded to an increasing prevalence of *Desulfovibrio* species in the bioreduction wells. 1/Simpson's diversity index were calculated by generating a distance matrix in MEGA version 3.1 and importing into DOTUR. Fluctuations in bacterial diversity were observed in the bioreduction wells while bacterial diversity continued to increase in the injection well.

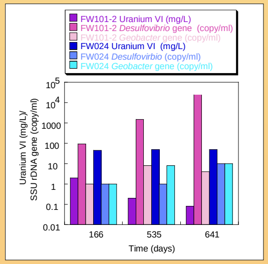

Figure 8. Determination of *Desulfovibrio* and *Geobacter* sequences. via q-PCR. In the bioreduction well, FW101-2, increasing *Desulfovibrio* sequences corresponded to declining uranium (VI) levels. The number of *Desulfovibrio* sequences were also 1000-fold higher in FW101-2 compared to FW024, a control well that was not stimulated. The number of *Geobacter* sequences did not change significantly over time.

DISCUSSION

➢ Bacterial community analysis of the wells at different times showed that the bacterial community was similar initially and developed changes over time with respect to the different phases of experimental manipulation.

➢ The injection well and the two inner bioreduction wells were stimulated for bacterial growth by using ethanol as the electron donor. These wells experienced changes in bacterial diversity, while that of the two outer wells displayed less change. The control well had a decline in bacterial diversity.

➢ Changes were also observed in the bacterial composition and structure and the physicochemical parameters of the wells throughout the bioremediation process. Initially, members of the denitrifying bacteria such as *Burkholderia* spp. and *Comomonadaceae* spp. were detected during the residual denitrification stage. During the initial phase of sulfate and uranium reduction, a few iron-oxidizer populations were observed, hence, indicating the importance of determining the factors that will improve the conditions for uranium reduction by sulfate and or metal reducers.

➢ The sulfate reducer, *Desulfovibrio* spp., and the metal reducer, *Geobacter* spp., were detected at the later phases of the bioremediation process where there were lowered uranium levels in each well. In particular, a predominance of the *Desulfovibrio* spp. population was observed. Conditions for uranium bioreduction was achieved.